CEB 2004 Bioaccessibility and Bioavailability Workshop

Assessing the Dermal Bioavailability of PAH from PAH-Contaminated Soils Using In Vitro Percutaneous Absorption Techniques

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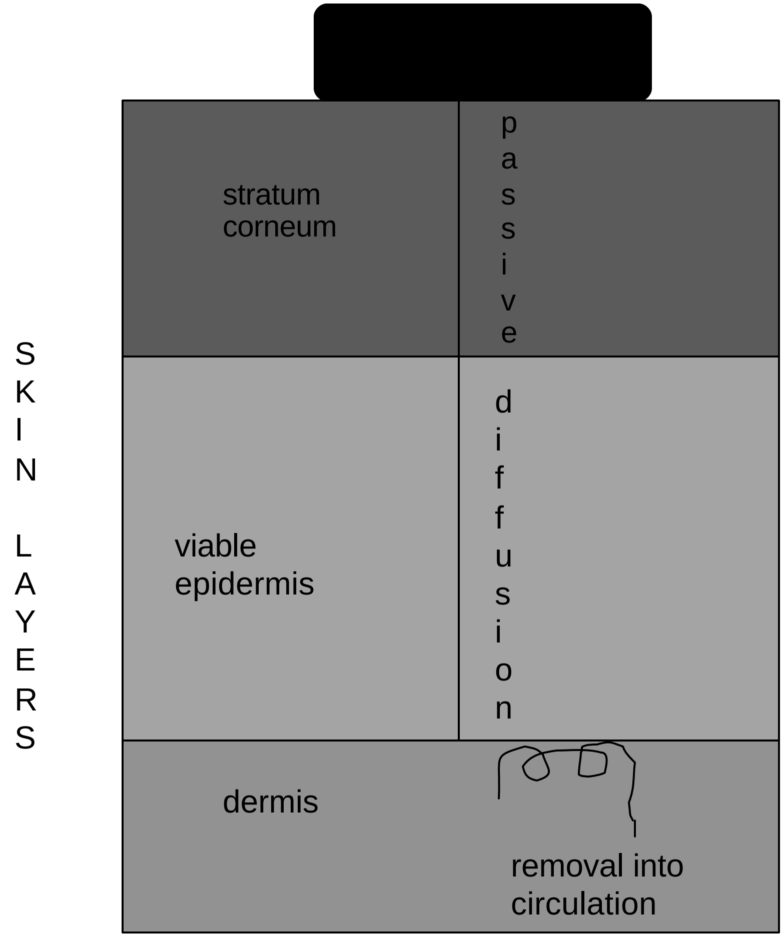
DISCUSSION OUTLINE

- ***In Vitro* Percutaneous Absorption – Method Development & Validation**
- **Application of the Method to Contaminated Soils - defining the variables**
- **Review of Lampblack Study**
- **Summary/Challenges**

Skin Schematic



Dermal Flux = J = Dk_p C/h

Where:

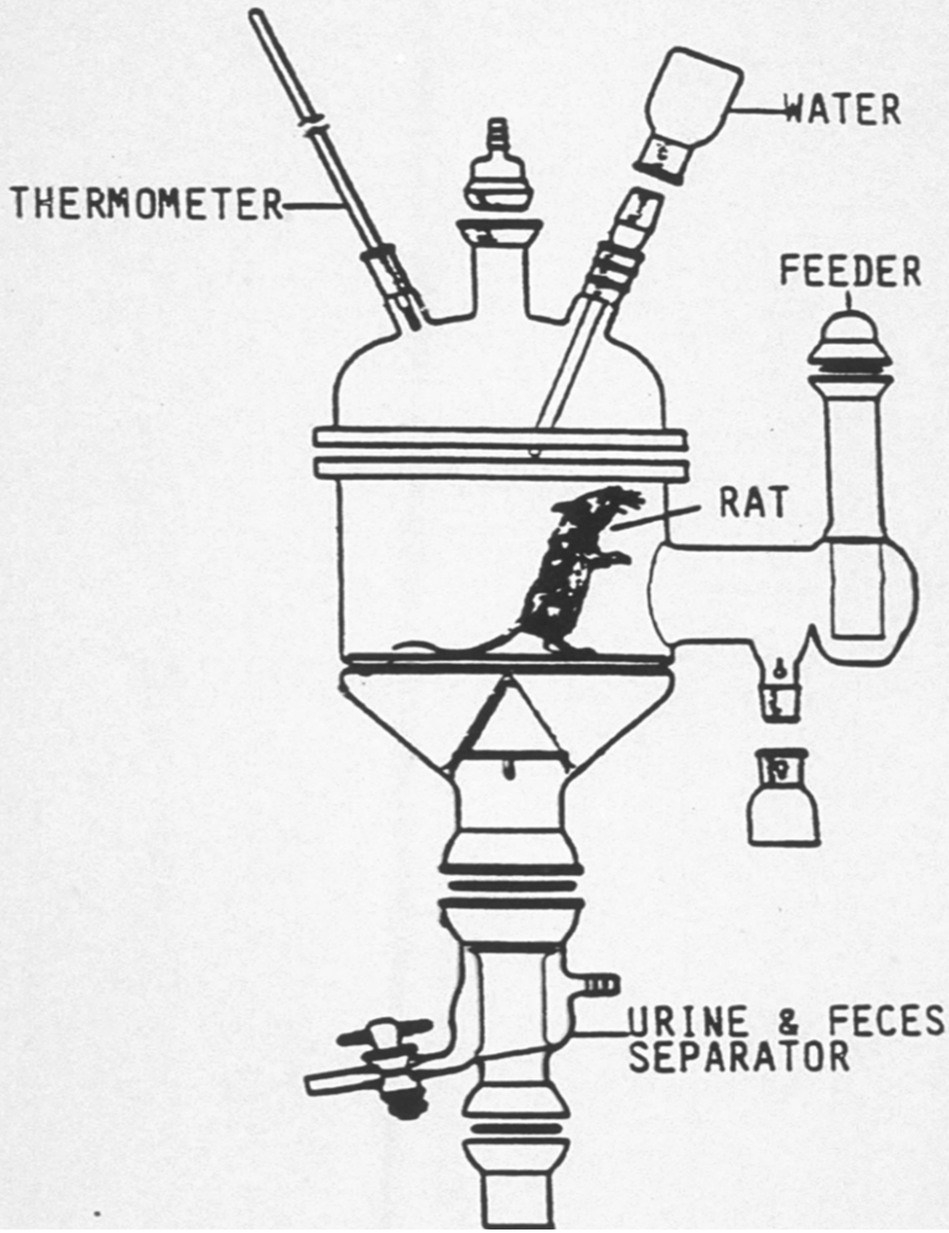
D = effective diffusion coefficient of chemical in SC

K_p = partition coefficient of chemical in vehicle

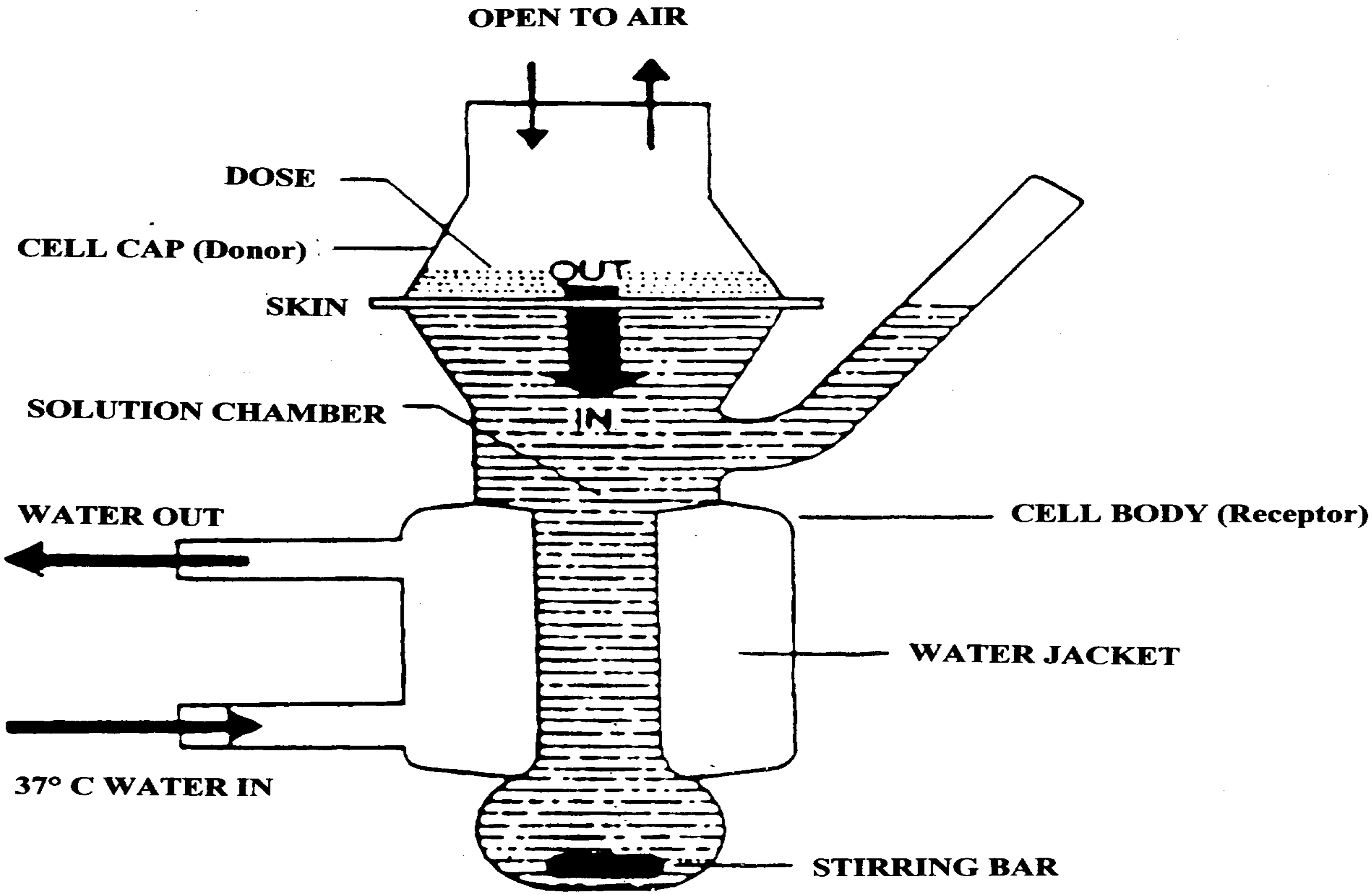
C = concentration of chemical in vehicle

h = effective diffusion path length through the skin barrier

<u>IN</u> <u>VIVO</u> METABOLISM CHAMBER.

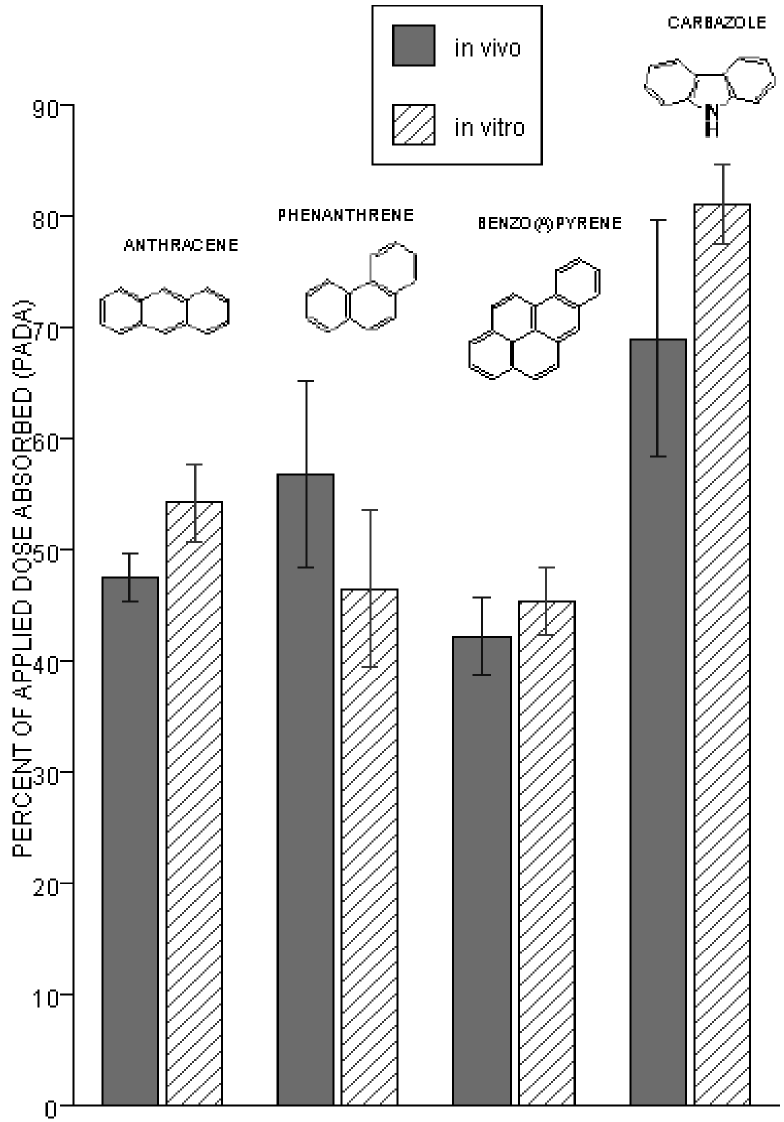



CAUTION
RADIOACTIVE
MATERIAL

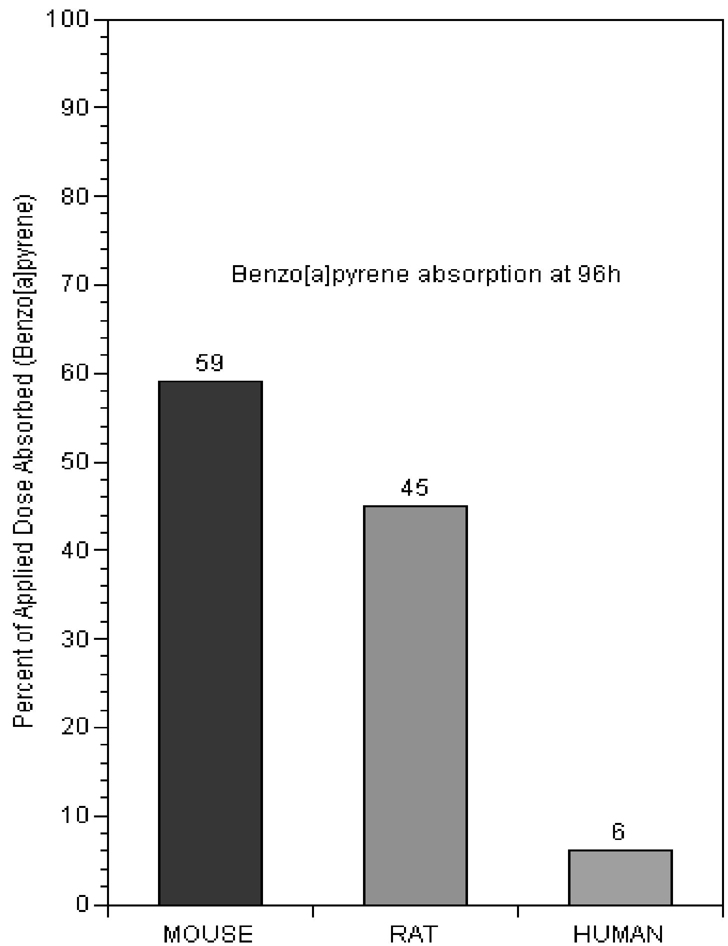


OPEN TO AIR
DOSE
CELL CAP (Donor)
OUT
SKIN
IN
SOLUTION CHAMBER
WATER OUT
CELL BODY (Receptor)
WATER JACKET
37° C WATER IN
STIRRING BAR

IN VIVO AND IN VITRO CORRELATION



IN VITRO PERCUTANEOUS ABSORPTION:
RODENT VS HUMAN

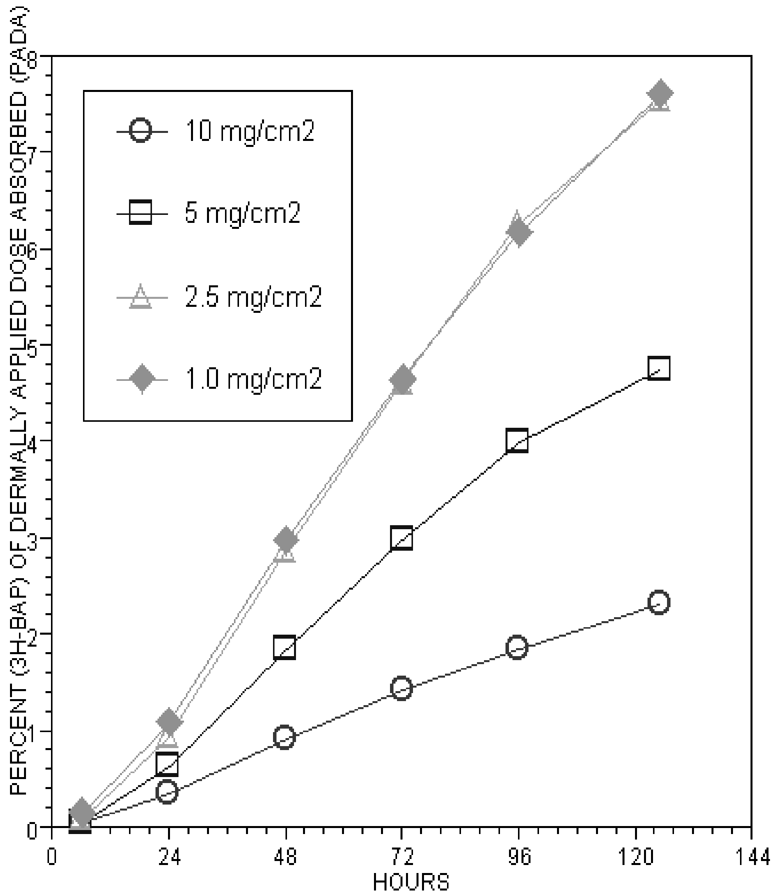


Summary of Percutaneous Absorption Guidelines

Protocol	Skin Type	Skin Preparation	Diffusion Cell	Receptor Fluid	Estimation of Absorption
Bronaugh & Collier (1991)	Viable or nonviable human or animal	Dermatome 200-350 µm; epidermis	Flow cell or static cell	Viable skin: physiological buffer, bovine serum albumin added for lipophilic compounds	Sum of receptor fluid and skin contents
EPA (1999)	Nonviable human	Dermatome 200-500 µm	Flow cell or static cell	Add 6% PEG 20 oleyl ether to increase solubility of lipophilic compounds	Determine permeability constant (Kp)
ECETOC[1] (2002)	Nonviable human or animal	Full or split thickness	Flow cell or static cell	Isotonic saline buffered to pH 7.4; surfactants and organic solvents added for lipophilic compounds	Determine Kp but skin levels also measured
ECVAM[2] (1996)	Nonviable human or animal	Full or split thickness	Flow cell or static cell	Saline, aqueous PEG or ethanol for lipophilic compounds	Usually measure just the receptor fluid
OECD[3] (2000)	Nonviable human or animal	Full or split thickness	Flow cell or static cell	Saline with solubilizers allowed for lipophilic compounds	Receptor fluid, but skin levels can be important

[1]ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals (Monograph)
[2]ECVAM = European Centre for the Validation of Alternative Methods (Report and Recommendations)
[3] OECD = Organization for Economic Co-operation and Development

Impact of soil loading on percent of applied dose (PADA)



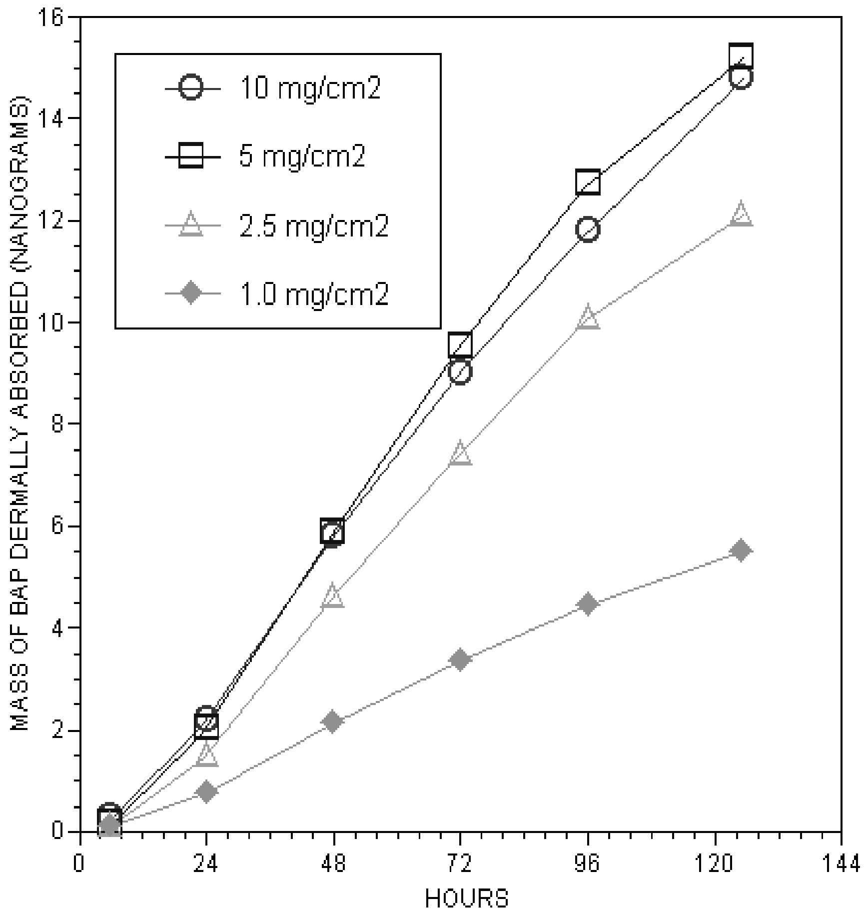
OBSERVATIONS:

BOTH THE 10 AND 5 MG/CM^2 COVERAGE CAN BE CONSIDERED “INFINITE DOSE” SITUATIONS - HALVING THE DOSE (10->5) DOUBLES THE PADA (2->4) WHICH ALSO SUGGESTS THAT ALL THE MATERIAL PARTIONING FROM SOIL TO SKIN IS CONTAINED IN THE MONOLAYER THE DATA CLEARLY SHOW THAT PADA HAS TO BE ADJUSTED TO SOIL COVERAGE.

OBSERVATIONS:

BOTH THE 2.5 AND 1.0 MG/CM^2 ARE LESS THAN MONOLAYER COVERAGE. THE DATA SUPPORT THE PREDICTION THAT PADA REMAINS CONSTANT AT SUB-MONOLAYER SOIL COVERAGE SINCE THE TOTAL MASS OF MATERIAL PRESENT DECREASES PROPORTIONATELY WITH DECREASING SOIL LOADING

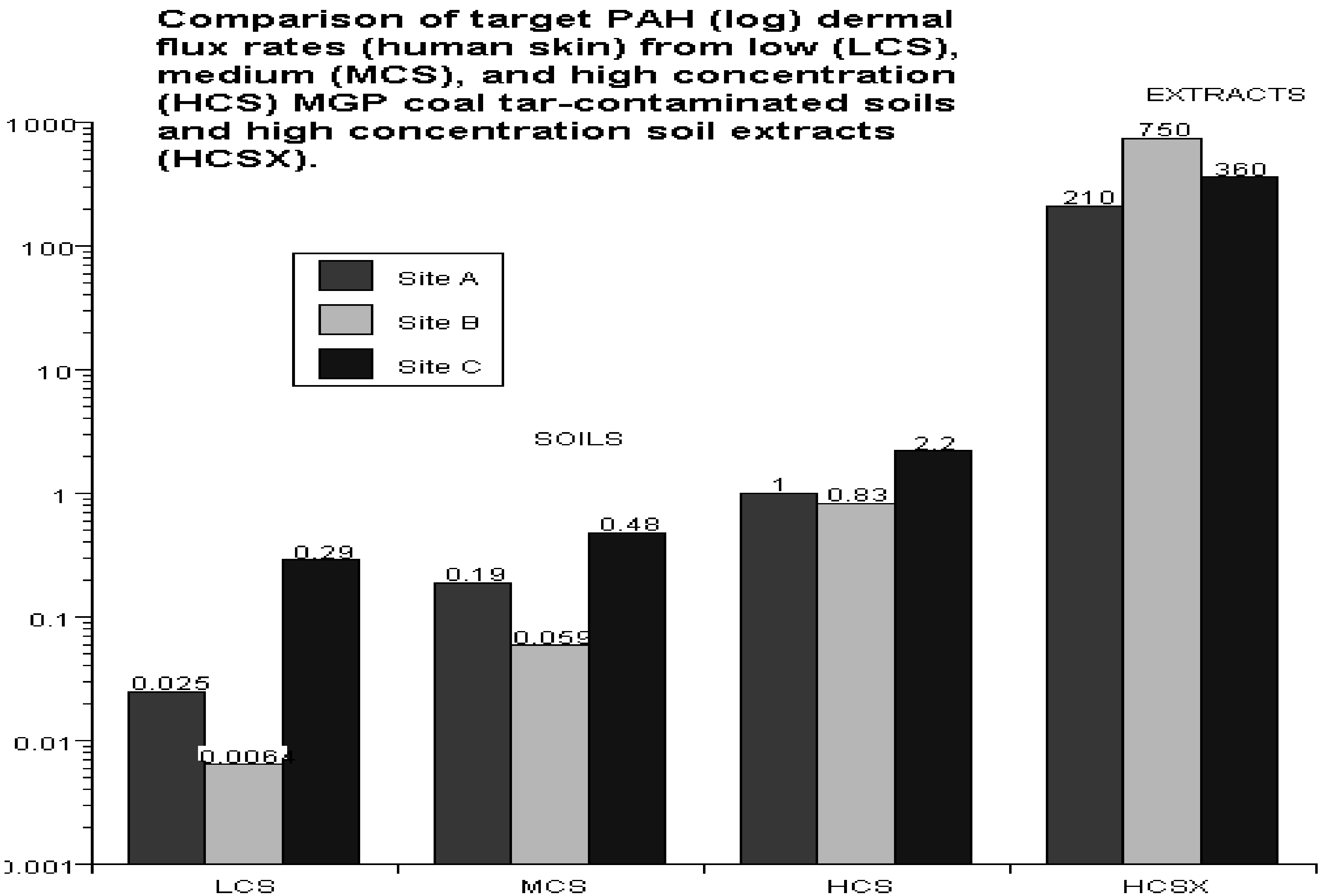
Impact of soil loading on dermal flux



OBSERVATIONS

- FLUX IS NOT AFFECTED BY SOIL LOADING ABOVE MONOLAYER (5 & 10 MG/CM^2)

- FLUX DECREASES IN PROPORTION TO SOIL LOADING BELOW MONOLAYER (1 & 2.5 MG/ CM^2)

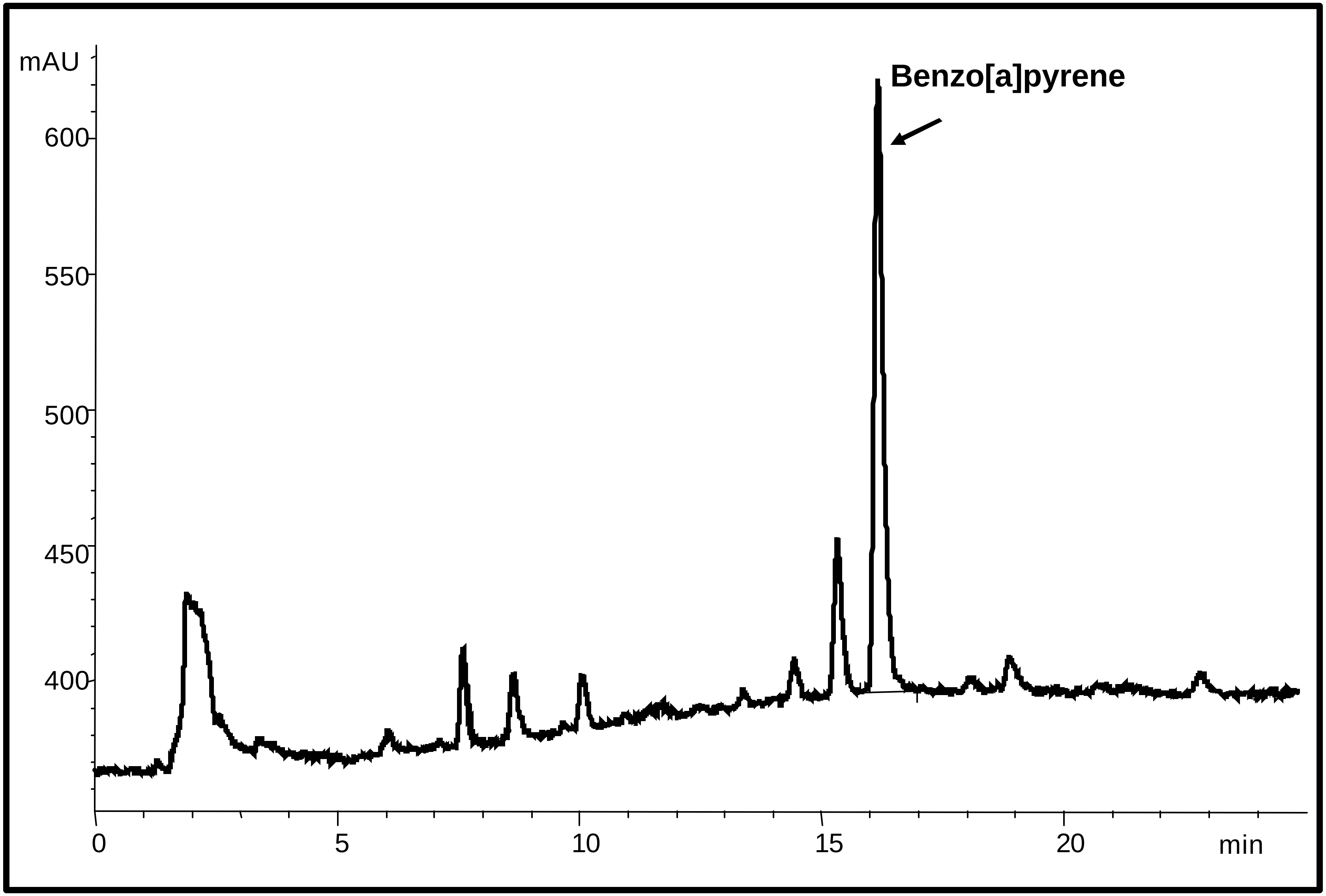

Comparison of target PAH (log) dermal flux rates (human skin) from low (LCS), medium (MCS), and high concentration (HCS) MGP coal tar-contaminated soils and high concentration soil extracts (HCSX).
EXTRACTS
SOILS
Site A
Site B
Site C
1000
100
10
1
0.1
0.01
0.001
0.025
0.0064
0.29
0.19
0.059
0.48
1
0.83
2.2
210
750
360
LCS
MCS
HCS
HCSX

OBSERVATIONS:

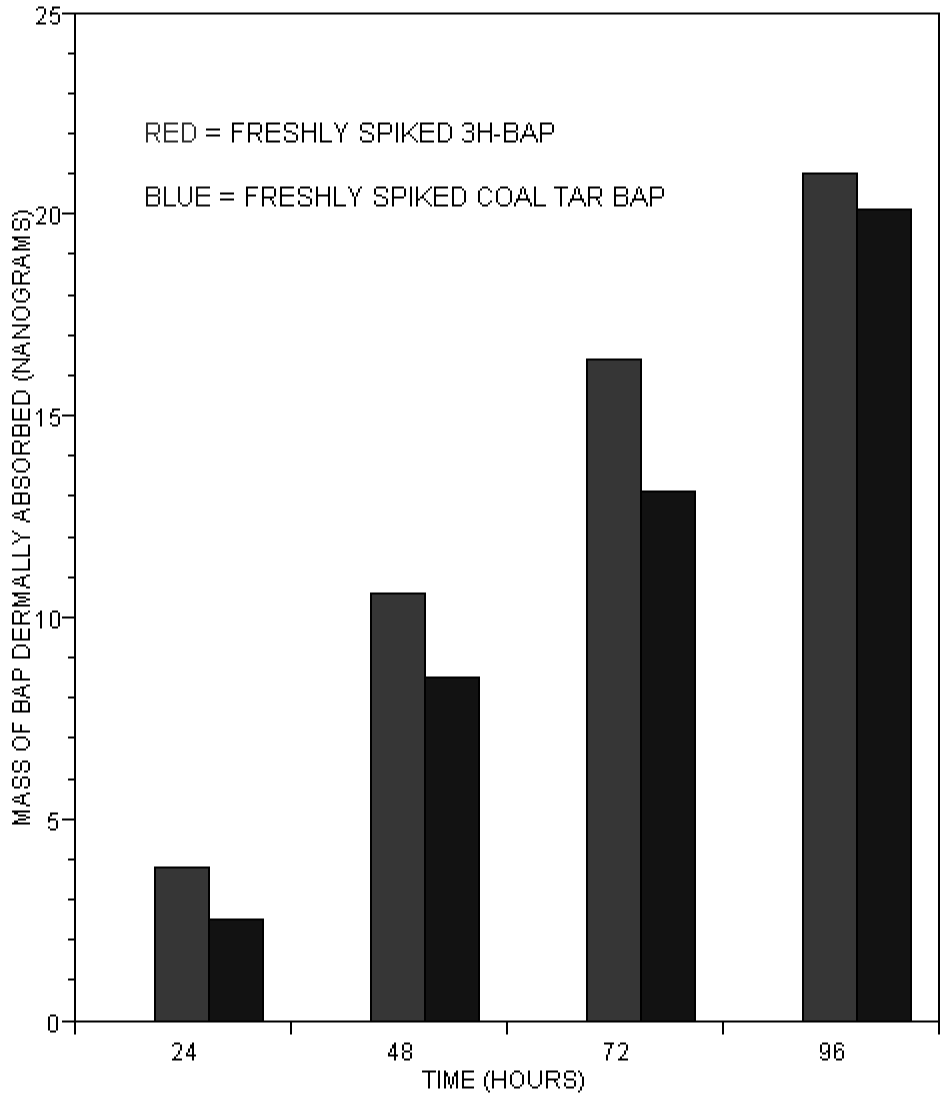
- SORPTION ON SOIL RETARDS THE DERMAL PENETRATION OF PAH BY A FACTOR OF 160-900

- SKIN PENETRATION RATE REDUCTIONS OF 10-30 CAN BE ATTRIBUTED TO SOIL BINDING EFFECTS ALONE

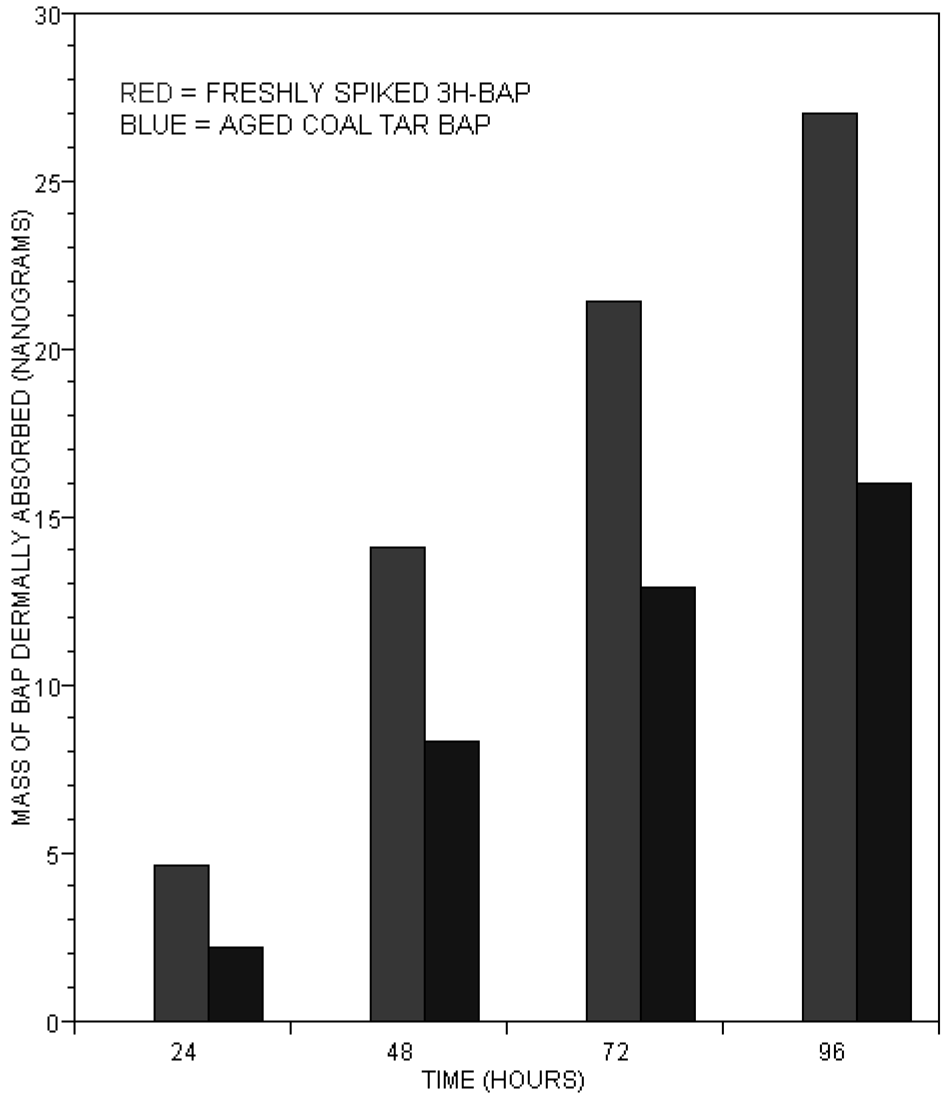
Chromatogram
BaP in receptor fluid (VOLPO-20)



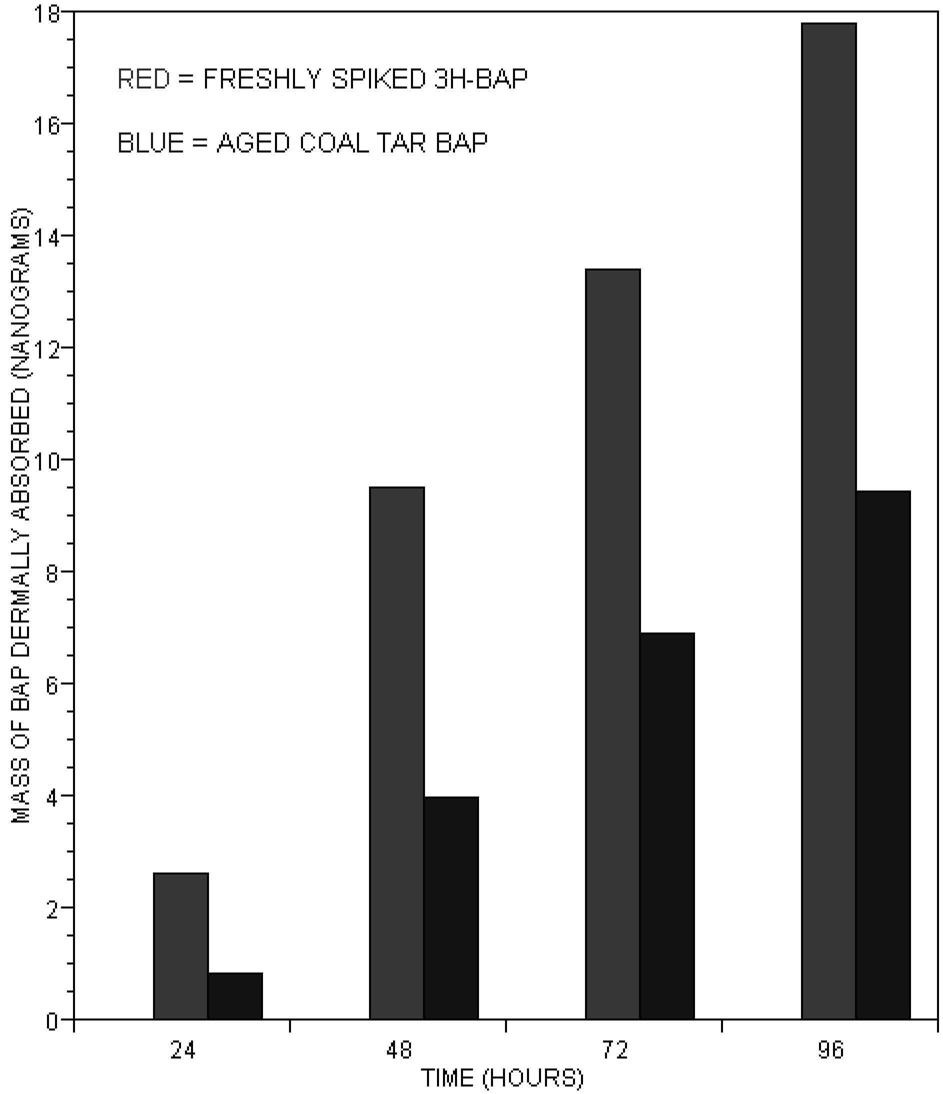
COAL TAR-CONTAMINATED SOIL AGING EXPERIMENT - **DAY 1**



COAL TAR-CONTAMINATED SOIL AGING EXPERIMENT - **DAY 45**



COAL TAR-CONTAMINATED SOIL AGING EXPERIMENT - DAY 110



OBSERVATIONS:

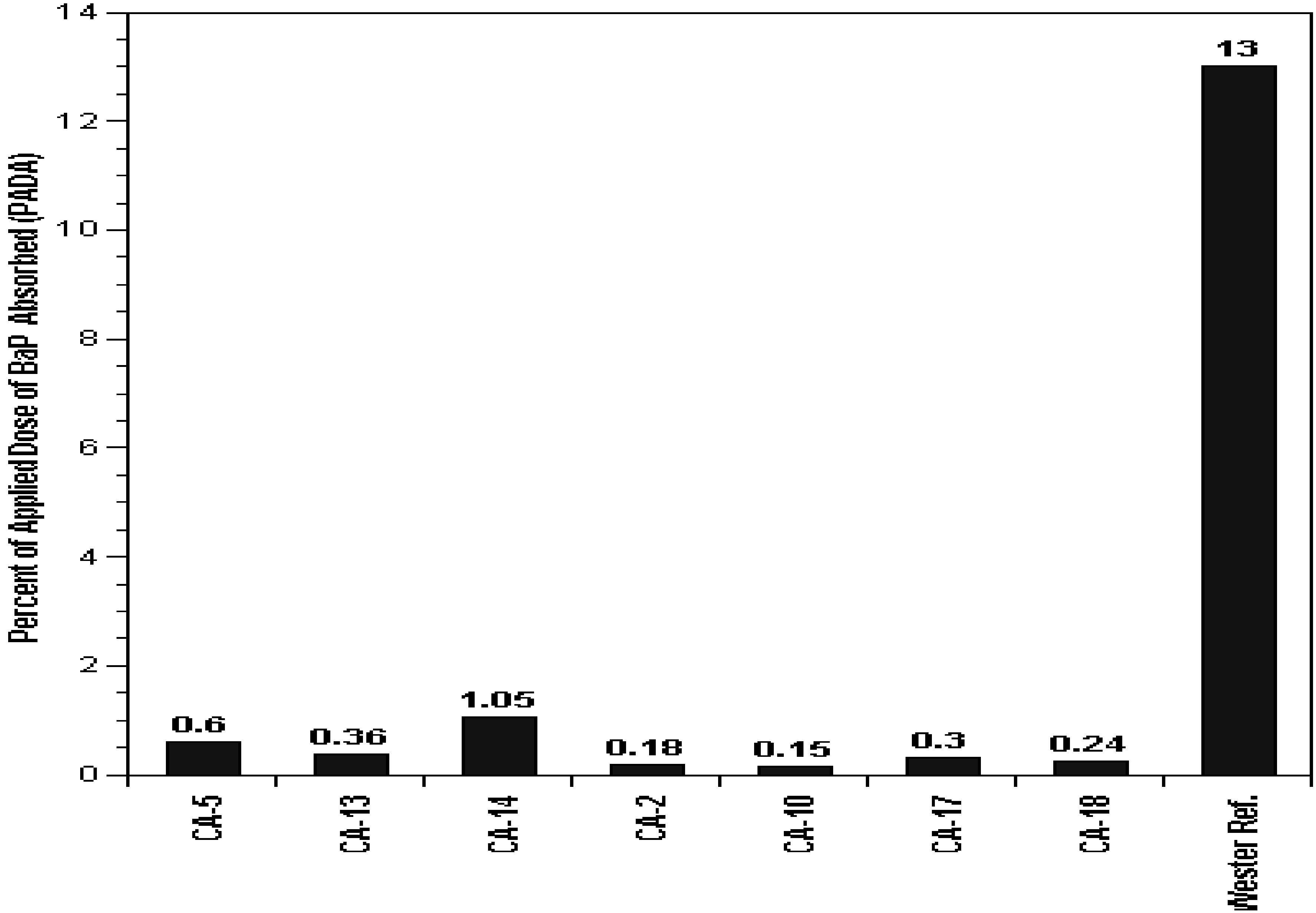
- LAST ON, FIRST OFF - THE “KINETICALLY” SORBED ^{3}H-BAP ACCURATELY REFLECTS THE FLUX OF “ENDOGENOUS” BAP IN THE COAL TAR-CONTAMINATED SOIL ON DAY 1

- AS A RESULT OF AGING, THE ‘ENDOGENOUS” BAP BECOMES MORE SEQUESTERED AND LESS DERMALLY BIOAVAILABLE (‘SLOW-RELEASE”). BY DAY 110, THE BIOAVAILABILITY OF THE ENDOGENOUS BAP IS ONLY HALF OF THAT FOR THE FRESHLY SPIKED ^{3}H-BAP

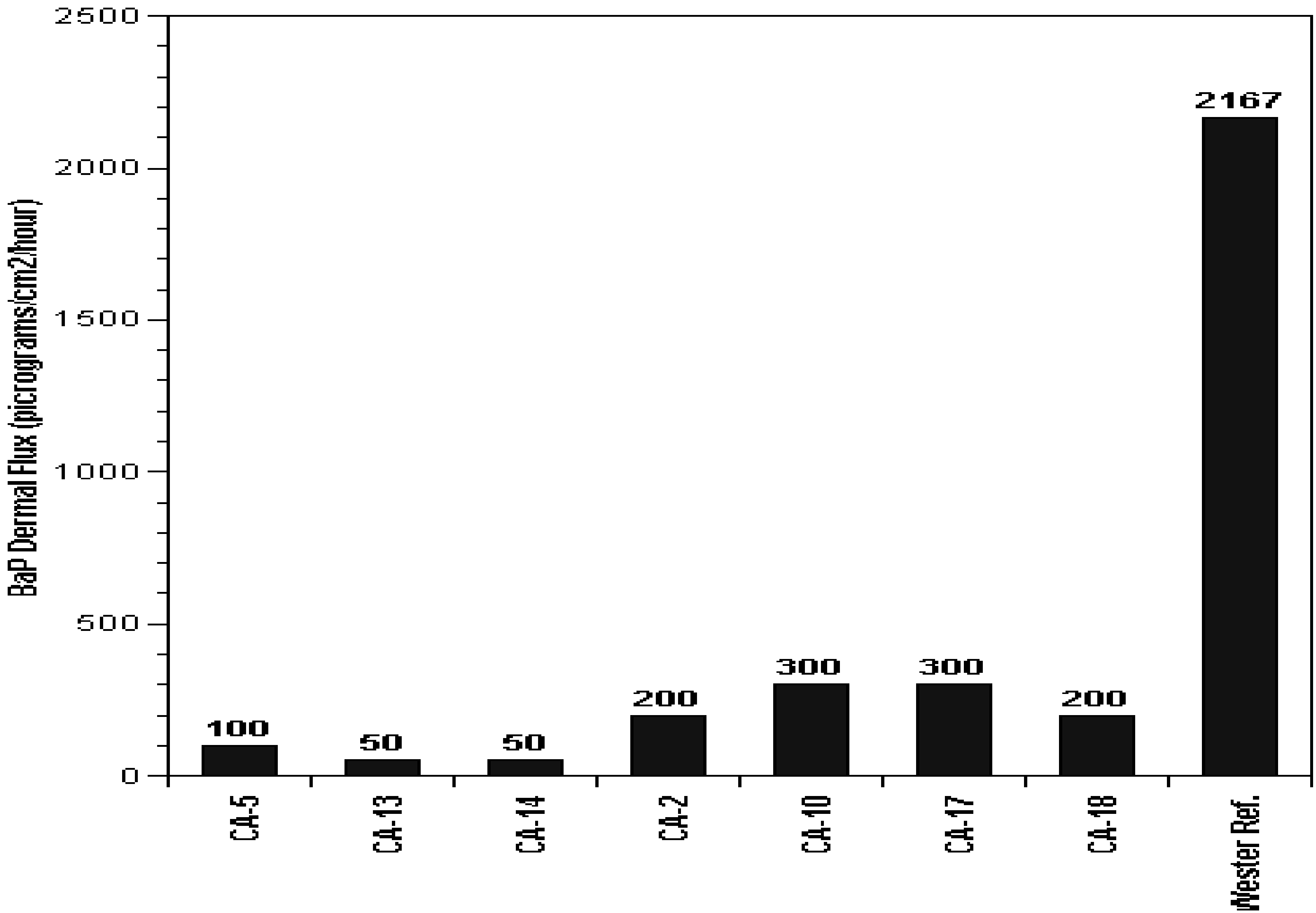
CONCLUSIONS:

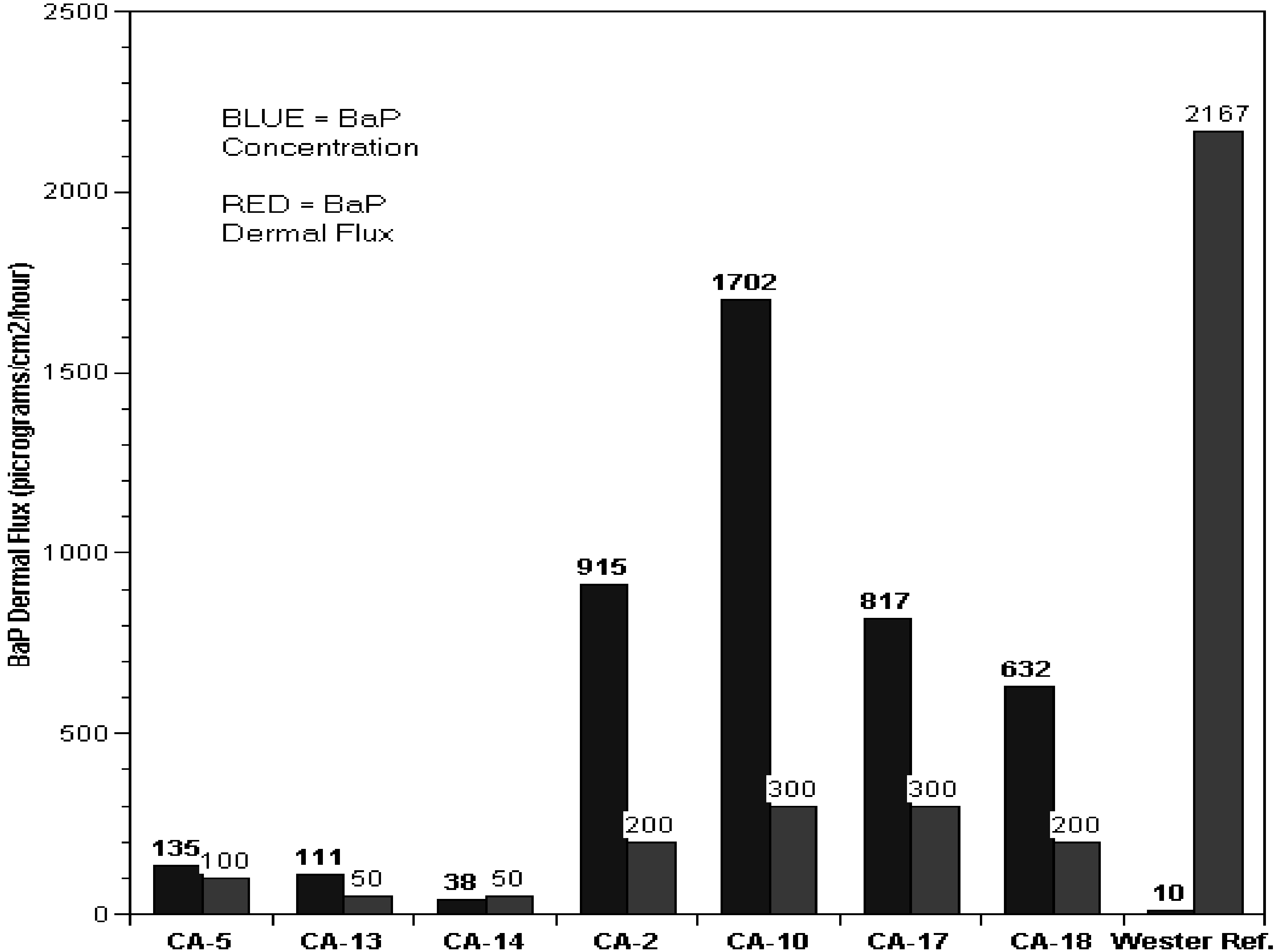
•PADA AND DERMAL FLUX (J) VALUES EXPERIMENTALLY DETERMINED AT "INFINITE DOSE" IN *IN VITRO* STUDIES CAN BE USED TO PROVIDE ACCURATE ESTIMATES OF PADA AND FLUX AT ANY SOIL LOADING, BOTH ABOVE AND BELOW MONOLAYER COVERAGE

•THE HPLC/FLUORESCENCE TECHNIQUE PROVIDES A DIRECT AND "NON-DISRUPTIVE" METHOD FOR MEASURING THE DERMAL BIOAVAILABILITY OF PAH-CONTAMINATED SOILS AND TO MEASURE THE IMPACT OF SOIL AGING ON PAH DERMAL BIOAVAILABILITY



Percent of Applied Dose of BaP Absorbed (PADA)
0
2
4
6
8
10
12
14
0.6
0.36
1.05
0.18
0.15
0.3
0.24
13
CA-5
CA-13
CA-14
CA-2
CA-10
CA-17
CA-18
Wester Ref.


BaP Dermal Flux (picrograms/cm2/hour)
2500
2000
1500
1000
500
0
100
50
50
200
300
300
200
2167
CA-5
CA-13
CA-14
CA-2
CA-10
CA-17
CA-18
Wester Ref.



BLUE = BaP Concentration
RED = BaP Dermal Flux
BaP Dermal Flux (picrograms/cm2/hour)
2500
2000
1500
1000
500
0
135
100
111
50
38
50
915
200
1702
300
817
300
632
200
10
2167
CA-5
CA-13
CA-14
CA-2
CA-10
CA-17
CA-18
Wester Ref.

Sample ID	Real and normalized [BaP]		B(a)P Flux ($pg/cm^2/hr$)	Flux normalized	Carbon Content (Wt%)[1]
CA-2	915	0.54	200	370	59
CA-5	135	0.079	100	1270	6.9
CA-10	1702	1	300	300	87
CA-13	111	0.065	50	770	6.5
CA-14	38	0.022	50	2270	2.9
CA-17	817	0.48	300	625	47
CA-18	632	0.37	200	540	25

- The r-value for soil BaP concentration vs BaP flux is 0.86
- The r-value for normalized flux vs carbon content is 0.71 (>90% significance at n=7)

OBSERVATIONS:

- Sorption on soil (lampblack) significantly decreases dermal bioavailability of PAH.
- The magnitude of PAH sequestering by “soils” is highly variable, dependent largely on SOC, but also, PAH concentration and aging – i.e., one size does not fit all!

CONCLUSION:

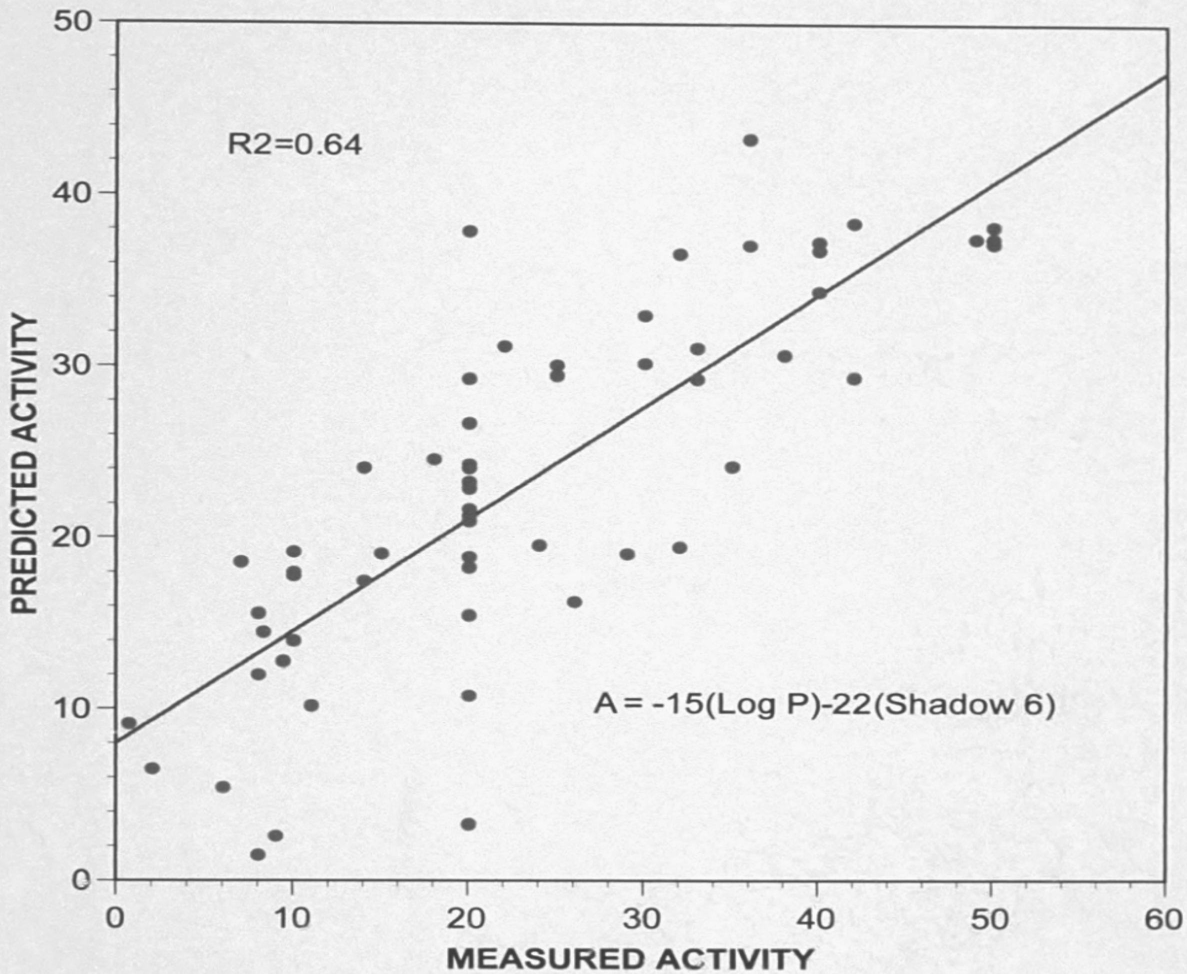
- In vitro dermal bioavailability studies based on internationally accepted experimental guidelines can provide site-specific data for realistic exposure and risk assessment.

CHALLENGES

(to researchers & regulators)

- Establish guidelines for conduct of *in vitro* dermal penetration studies with soils
- Accept the fact that soil is not water and is too complex a matrix to fit into a universally applicable model to approximate delivered dose (- site specific data matters!)

Plot of predicted versus measured activity for N=60 PAH



$$DAD = DA \times EF \times ED \times A/(BW \times AT)$$

where:

- DAD = dermally absorbed dose (mg/kg/day)
- DA = dose absorbed per exposure (mg/cm^2/8-hr day)
- EF = exposure frequency (350 days/year)
- ED = exposure duration (30 years)
- A = exposure surface area (2000 cm^2 - head & hands)
- BW = body weight (70 kg)
- AT = average time (25,550 days over 70 years)

$$\text{Cancer Risk} = 1 - \exp(-DAD \times q^*)$$

$$\text{Hazard Index for Non-Cancer Effects} = DAD/RfD$$

where:

- q^* = 95% upper-confidence limit of the linear-slope factor
- RfD = Reference dose